

Review Article

QUANTITATIVE ANALYSIS OF BIOLOGICAL NITROGEN FIXATION IN VARIOUS MODELS OF LEGUMES AND THE FACTORS INFLUENCING THE PROCESS: A REVIEW

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ABSTRACT

Biological nitrogen fixation refers to the biological reduction of dinitrogen gas to ammonia under normal temperature and pressure. Leguminous crops can combine this abundantly present gas of the atmosphere, which has proven to be beneficial for both the crops themselves and any other succeeding crops growing in the same soil, thereby downsizing the utility of nitrogenous fertilizers. Several techniques are available for the direct quantitative measurement of legume biological nitrogen fixation in the field and controlled environments. However, these are time-consuming and therefore, costly and generate data relevant only to the time and place of measurement. As an alternative, legume biological nitrogen fixation is calculable by either empirical models or dynamic mechanistic simulation models. Comparatively, simulation by a dynamic model is calculable for quantifying legume biological nitrogen fixation, because of its capability to simulate the response of N fixation to a good vary of environmental variables and legume growth standing presently. In this review, an attempt was made to discuss and compare the strategies used to estimate the potential N fixation rate, and therefore the response functions to simulate legume biological nitrogen fixation in nine widely cited models over the last thirty years. Further, assessing their relative strength in simulating legume biological nitrogen fixation with varying biotic and abiotic factors and determines the discrepancies between experimental findings and simulation was carried out. In conclusion, this review clarifies, the current progress in legume biological nitrogen fixation quantification in simulation models, and guides their development, combining elementary experimental and modelling work.

Keywords: Leguminous plant, Nitrogen fixation, Nitrogen-fixing bacteria

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INTRODUCTION

Nitrogen is present in the atmosphere in its molecular form. In this conformation, it comprises of two nitrogen atoms bonded to each other by a triple bond. This contour of nitrogen is inert. To be biologically active the nitrogen needs to combine with other atoms to form compounds like ammonia (NH₃) or nitrates (NO₃) that can be utilized by living organisms for the conglomeration of vital biomolecules like proteins and DNA. The chemically inert nitrogen gas of the atmosphere can be converted into a biologically active form by the course of biological nitrogen fixation. Thus, biological nitrogen fixation may be defined as the process by virtue of which the inert molecular nitrogen gas present in the atmosphere is converted to a biologically usable form in the soil with the aid of microorganisms. Nitrogen fixation refers to the combination of the inert gas with other atoms, generally leading to either oxidation of the gas-producing nitrates or reduction of the gas-forming ammonia. The reaction involves breaking of the triple bond between the nitrogen atoms with nitrogenase enzyme acting as a catalyst along with the aid of 8 electrons and 16 ATP molecules, ultimately leading to the production of ammonia and hydrogen gas. Biological Nitrogen Fixation can hence be considered as the first step of the Nitrogen Cycle. Nitrogen (N) applied in fertilizers or manure that are not concerned by crops will be free into the atmosphere as greenhouse gases [1] or leached into groundwater [2, 3], with resulting environmental implications.

The scientific discipline utilization of biological nitrogen fixation contains a long history. In biological science and agroforestry, nitrogen-fixing utile trees have become vital and additional research is required to optimize their use. The field contains a long tradition of applied biotechnology using legume inoculation for enhanced nitrogen fixation and yields. Legumes are well known for their rhizobial symbiosis that introduces nitrogen into the global nutrient cycle [4]. There are many techniques available for measuring legume biological nitrogen fixation in the environmental field [5, 6]. Models

can test how legumes can be used to meet environmental and production goals [7]. These strategies can reveal the response of N fixation to variable factors in real things; however, they are restricted to the conditions prevailing at the time of measure and can't be accustomed to predict N fixation. Thus, there is a desire for strategies to predict legume biological nitrogen fixation quantitatively to assist build choice concerning developing and managing property farming schemes. Estimating N fixation from crop yield or empirical models supported historical crop knowledge will be used; however, these are restricted to a selected site for an equivalent crop. Modeling is probably an improved tool to grasp and quantify legume biological nitrogen fixation because it relies on data of the mechanisms concerned, climate and management, and might accurately represent variations in legume biological nitrogen fixation beneath totally different things. A further complication is that the mathematical functions accustomed to simulate legume biological nitrogen fixation vary between models. For example, the rate of potential N fixation is estimated either by plant N demand and uptake [8, 9], nodule biomass [10, 11], root biomass [12, 13] or aboveground biomass [14, 15]. Also, the response of N fixation to soil mineral N concentration in the root zone, which is closely correlated to N fixation and thus the benefit to soil N fertility from legumes [16], differs between models.

Nitrogen fixation is either very sensitive [11, 17] or tolerant [18, 19] to high soil N concentration in different models [8]. In this paper, we tend to elaborate on the modeling methods published for quantifying legume biological nitrogen fixation by grain and forage legumes. Additionally, to distinguish the different schemes and functions used to simulate the response of N fixation to organic and analyze their relative strengths and weaknesses was reviewed. Further, an attempt was made to determine gaps in the current models and create suggestions to boost the simulation of legume nitrogen fixation in future models. A systematic search was carried out in Pub Med, Scopus and Web of Sciences using a combination of Boolean operators. Peer-reviewed papers in English on the

keywords related to biological nitrogen fixation were retrieved and evaluated based on titles and abstracts. The retrieved papers were managed using Mendeley and the data were consolidated.

Environmental parameters regulating biological combination of nitrogen in leguminous plants

Advance method of legume biological nitrogen fixation is stricken by environmental conditions like temperature, water content, N concentration, root zone hydrogen ion concentration, plant nutrient standing together with C and N substrates in roots, and genetic variation in potential N fixation capability. It is conjointly stricken by organic plant process standing like phosphorus (P) and a metallic element (K) levels that management nodule growth and enzyme activity directly or indirectly [20]. During this article, we tend to focus solely on however legume biological nitrogen fixation responds to environmental conditions and plant C and N substrates intending to improve models. Although here, we tend to only elaborate on this subject in broad terms, several recent review articles cover this space in a lot of greater detail [21]. As may be wanted from the quantity of genes concerned, the consecration and restraint of bacterial nodulation genes are under tight regulative management and could be a major issue affecting host specificity and retort to environmental parables [22].

Soil temperature

Generally, the heat content of the soil prevents the ability of Leguminosae members to biologically combine nitrogen by exercising its control on nodulation, nodule establishment, and nitrogenase activity once it is either too lofty or too crouched [23, 24]. Temperature subsumes salient clout on the endurance and tenacity of rhizobial strains in soil. Soil temperature within the root zone is one amongst the dominant factors for nodulation and establishment. For instance, the nodulation of arrowleaf clover (*Trifolium vesiculosum* Savi.) is increased at a root temperature of 25 °C compared there with growing at each 18 °C and 32 °C [25].

However, the retort of nodule initiation to soil temperature alters between species and varieties. In soybean (*Glycine soap* (L.) Merr.), a lot of nodules square measure created within the pristine extension phase at 25 °C, whereas 20 °C is perfect for nodule length once nodule procreation is concluded as to 15 °C and 30 °C [26]. In distinction, nodule formation is increased with augmenting temperature within the range of 10–35 °C for trefoil (*Trifolium repens* L.) no matter the varieties and, therefore the rhizobia strains [27, 24].

There is an outsized variety of examination on the riposte of N fixation in temperature in legume crops, scruple temperatures for N fixation dissent amidst species from 2 °C to 10 °C, and usually tropical and subtropics legumes have surpassed scintilla temperatures than temperate species. Nitrogenase enterprise is tremendously around 12–35 °C and reaches most at 20–25 °C in most legumes. N fixation in subterranean trefoil (*Trifolium subterraneum* L.) is extremely active at a good vary of temperatures, from 5 °C to 30 °C; however, ebbs persuasively with cold and nearly ceases at 2 °C. Generally, 35 °C or 40 °C is that the higher restraint of temperature for legume biological nitrogen fixation found that out of a hundred rhizobia of untamed soybean in yellow river delta soils in China, four strains exhibited sturdy tolerance to acidity, pH scale, and salinity, as well as high and low temperatures and conjointly had sturdy nodulation capability [28]. Temperature features a profound influence of N₂ metabolism. Little activity is ascertained by vasoconstrictive and warming promotes the microbial N₂ fixation and uptake of mounted gas [29].

Soil water enterprise

In a congruous manner to soil temperature, soil water content within the root zone clouts N fixation over nodule initiation and nodule activity, and gas permeability [30-33]. Soil water paucity impedes N fixation [24, 34], and also, the reticence is invigorated as drought stress becomes a lot more fervent [35]. Besides, water-logging will seriously scale back N fixation over the abasement of the initiation and exercise of nodules [20]. It is tasking to appraise the connection amongst N fixation and soil water standing exactly, thanks to the

restrictions of experimental regulates. Stress periods and plant recovery [36, 37]. A sigmoid expression has been used to describe the retort of N fixation by common bean (*Phaseolus vulgaris* L.), black gram (*Vigna mungo* (L.) Hepper and cowpea (*Vigna unguiculata* L.) Walp to soil water stress, illustrating acute senility in N fixation as soil water stress becomes more resolute [38, 14].

Carbon requirement at different growth stages for biological Nitrogen fixation

Photosynthate divide to roots supports nodule growth, provides energy for N fixation, maintains a useful population of rhizobia, and permits the synthesis of amino compounds produced from N fixation [39-41]. The C value of N fixation also varies with growth stages [42, 43], but it is a matter of discussion that the C value will increase [44, 45] or decreases [46] with the course of the legume life cycle. Additionally, the strain of the genus *Rhizobium* could affect the C value considerably.

Periodic changes of biological nitrogen fixation in legume

The amount of legume biological nitrogen fixation changes with physiological growth factors. It is low within the early growth stages, whereas nodules are establishing [47] and reaches the most worth between early flowering and early seed-filling, depending upon the species and growing conditions [48-51]. The ability of legumes to form nodules has been attributed to many soil and biological factors that include levels of mineralizable N, levels of available P, soil reaction in the form of pH, type and vigour of legume [52].

Quantification of legume biological nitrogen fixation

Legume biological nitrogen fixation is also quantified by direct measuring, estimation supported yield or with empirical models or simulation of crop models. The ways accustomed live N fixation directly to this point, like the acetylene reduction/hydrogen increment assay, N distinction, 15N-labelling, and ureide, has been completely reviewed [6, 7]. The review of those ways is on the far side of our focus, and during this paper, we tend to describe and compare methods solely to estimate and simulate N fixation.

Crop growth model

Crop growth models are the computer software system programs that may simulate daily growth (e. g. biomass, yield) and development of crops like wheat, maize or potato. Reckoning on the soil characteristics, climatic conditions and crop species, crop models calculate the daily growth of biomass within the individual plant organs (stem, leaves, roots, grains, etc.) moreover because of the progress of plant development from the sowing to maturity. Additionally, crop model accounts for vital processes within the soil (water and nutrient availability) to simulate crop growth throughout a full season. Furthermore, associate advance model, because the one utilized in, FARM/IT calculate nitrogen mineralization, activity volatilization reckoning on precipitation and soil wetness content. Nitrogen deficiency and drought stress method scales back crop biomass growth and yields. Crop models, like DSSAT-CSM batch and APSIM, are enormously used in examining, estimation, and the prophecy of crop growth and manufacture, on-site step up to topical or national levels.

Analysis with empirical models

Static estimation of N fixation throughout the total growing season could use either economic yield or above-ground dry matter. The proportion of legume if it is intercropped, N derived from N₂ fixation and the magnitude relation of the existed N belowground to the fixed N surface. As an alternative, the proportion of fixed N transferred to a relative crop, consumed by grazing animals or lost by immobilization [53]. The second methodology to estimate N fixation is by empirical models supported the correlation of fixed N within the final yield against variables, like harvested dry matter or the proportion of legume in mixed leys. A linear equation has been fitted to the measured information of mixed trefoil and grass swards at completely different sites from four countries. This showed a big correlation between fixed N and also the further dry matter of mixed leys, compared with corresponding pure grass either to cut sward or grazed sward [54]. Kristensen *et al.* [55] established that combined

nitrogen at reap exaggerated precariously with clover dry matter content in the mixed meadow through statistically examining the provisional information from completely different sites with distinct soil sorts and irrigation schemes. The first time an immediate estimation of N fixation, and therefore the parameter values are quite simply measured on-site or calculable from the literature. It doesn't strictly need to associate an adequate dataset for multiple years to work out the parameters. Therefore it is simple to use. However, when determining the parameter values, knowledge from years of abnormal weather ought to be avoided, and therefore the properties of the soil should be comparatively stable year-on-year. As these equations are independent of environmental factors like soil properties and climatic conditions, they are solely applicable and correct for similar sites and average climatic conditions.

Additionally, the parameter values ought to be adjusted if the equations are used for various sites or legumes. In contrast, the second technique relies on statistical correlation and assumes that N fixation contains a strong linear relationship to the variables. It is a lot of intensity to use and might be applied to one specific site or multiple sites with completely different soil varieties, counting on however the empirical relationship is developed and which sites the information was obtained from. However, like the first technique, these approaches are restricted to specific sites as a result of the equation is not ready to represent the interaction between plant and environment mechanistically.

Legume simulation in a crop growth model

The simulation of legume biological nitrogen fixation in soybean developed by [56] Duffy *et al.* [56], searching into the biophysiochemical transformations of N in tile-drained soil, may well be the earliest mechanistic model involving leguminous N fixation. The rate of N fixation by soybean within the model depends on the basic

growth rate. In more recent simulation models of biological nitrogen fixation in legumes, the most popular methodology to estimate the speed of legume and biological nitrogen fixation in a potential or most fixation rate changed by the influence of environmental factors. The potential fixation rate is estimated to support either a demand-uptake mechanism or on the dry matter of plant tissues and is varied with plant growth stages. Environmental factors commonly embrace soil temperature, soil or plant water content, soil mineral N or substrate N concentration in plant tissues, and substrate C concentration in the plant. Alternative factors, like soil pH, salinity and also the supply of alternative nutrients, haven't been enclosed in models to date. In this paper, we tend to review the most-used recent simulation models within which a legume N fixation performance has been enforced.

Dissimilitude in erosion productivity impact calculator (EPIC) and agricultural production systems simulator (APSIM) models

EPIC and APSIM models use variations of the primary definition to estimate the potential N fixation rate. The EPIC model assumes that the entire plant N demand is up to the potential N fixation [8, 9]. APSIM defines essential N concentrations for plant tissues and uses these to estimate N demand by maintaining non-stressed N levels in plant tissues and supporting the N demand of recent tissues. APSIM is a biogeochemical model that can simulate productivity and greenhouse gas emissions. The model uses a scheme inspired by DayCent to calculate the fraction of total nitrification and denitrification that is emitted as N₂O [57, 58]. N fixation is just calculated if N uptake can't meet the plant N demand. Therefore, the potential N fixation is assumed to be the difference between plant N demand and N uptake [6, 15]. The second definition relies on the sturdy relationship between N fixation and either nodule size/biomass [31, 45].

Table 1: Simulation models that indulge legume biological nitrogen fixation

Model	Simulated legume species	Reference
Sinclair Model	Soybean	[14]
Erosion Productivity Impact Calculator (EPIC)	Soybean, cowpea, black gram	[9, 18, 19]
Hurley Pasture Model	White clover	[12, 60, 61]
Schwinning Model	Field pea White clover	[17, 62]
Crop Growth Model (CROPGRO)	Soybean, peanut, dry bean, velvet bean, faba bean, cowpea	[63-65]
SOILN	White clover	[6, 15]
Agricultural Production Systems Simulator (APSIM)	Soybean, chickpea, peanut, mungbean, lucerne	[11]
Soussana Model	White clover	[13]

In addition to direct field measurements, estimates of legume biological nitrogen fixation are based on harvested yield or are derived from easy empirical models. Simulation of legume biological nitrogen fixation by models that incorporate the dynamics of N fixation might be the most effective approach as they will facilitate the North American nation to know the nature of the elaborate relationships between N fixation and environmental and plant factors. Our review has found that the tactic used to simulate legume biological nitrogen fixation most often in recent publications is to change potential N fixation rate by factors like soil temperature, soil/plant water, soil/plant N, plant C, and plant growth stages. Despite this variety of approaches, the simulation of legume biological nitrogen fixation in recent models could also be handily summarized as the influence of soil temperature on legume biological nitrogen fixation is often described by a four-threshold-temperature operate, or a sigmoid cubiform perform within the Hurley Pasture Model [59]. The values of those four threshold-temperatures vary with species and vascular plants. Besides, they clarify soil salinity on the vascular plant.

CONCLUSION

This review critically interprets the ways accustomed quantify legume biological nitrogen fixation by the foremost usually used experimental and modelling approaches. To simulate legume biological nitrogen fixation by totally different models and assesses their relative strength in predicting nitrogen fixation with variable biotic and abiotic factors. The stimulating impact on legume Biological nitrogen fixation at comparatively low levels of soil

mineral N ought to be distinguished from the inhibition of legume biological nitrogen fixation by soil mineral N. The simulated inhibition of legume biological nitrogen fixation by soil mineral N could be exaggerated if potential N fixation is changed by functions that embrace nodule biomass and therefore the impact of soil mineral N on potential N fixation. More experimental work is required to characterize the result of each soil water deficit and excess soil water on biological nitrogen fixation legume. The responses of legume biological nitrogen fixation to alternative factors presently absent from all models, like soil pH scale and O₂ porousness, have to be compelled to be enclosed and reinforced with adequate experimental work. Intercropping of legumes either with grain crops or in grasslands, because the presence of grazing farm animals affects legume biological nitrogen fixation within fields. Models of legume biological nitrogen fixation should take a higher account of these vital practical uses of legumes.

AUTHORS CONTRIBUTIONS

All the authors have contributed equally

CONFLICT OF INTERESTS

Declared none

REFERENCES

1. Flechard CR, Ambus P, Skiba U, Rees RM, Hensen A, Van Amstel A *et al.*, Effects of climate and management intensity on nitrous

- oxide emissions in grassland systems across Europe, *Agric Ecosyst Environ.* 2007;121:135–52.
2. Stout WL, Fales SL, Muller LD, Schnabel RR, Weaver SR. Water quality implications of nitrate leaching from intensively grazed pasture swards in the northeast US, *Agric Ecosyst Environ.* 2000;77:203–10.
 3. Trindade H, Coutinho J, Jarvis S, Moreira N. Nitrogen mineralization in sandy loam soil under intensive double-cropping forage system with dairy-cattle slurry applications, *Eur. J. Agron.* 2001;15:281–93.
 4. Pfau T, Christian N, Masakapalli SK, Sweetlove LJ, Poolman MG, Ebenhoh O. The intertwined metabolism during symbiotic nitrogen fixation elucidated by metabolic modelling. *Sci Rep.* 2018;8:12504.
 5. Goh KM, Bruce GE. Comparison of biomass production and biological nitrogen fixation of multi-species pastures (mixed herb leys) with perennial ryegrass-white clover pasture with and without irrigation in Canterbury, New Zealand, *Agric Ecosyst Environ.* 2005;110:230–40.
 6. Herridge DF, Peoples MB, Boddey RM. Global input of biological nitrogen fixation in agricultural systems, *Plant Soil.* 2008;311:1–10.
 7. Carlsson G, Huss-Danell K, Dakora FD, Chimphango SBM, Valentine AJ, Elmerich C, Newton WE (Eds.) How to quantify biological nitrogen fixation in forage legumes in the field, in: *Biological Nitrogen Fixation: Towards Poverty Alleviation through Sustainable Agriculture*, Springer Netherlands, Dordrecht 2008;pp. 47–8.
 8. Fitton N, Bindi M, Brilli L, Cichota R, Snow V. Modelling biological N fixation and grass-legume dynamics with process-based biogeochemical models of varying complexity. *Eur J Agro.* 2019;106:58–66.
 9. Cabelguenne M, Debaeke P, Bouniols A. EPIC phase, a version of the EPIC model simulating the effects of water and nitrogen stress on biomass and yield, taking account of developmental stages: validation on maize, sunflower, sorghum, soybean and winter wheat. *Agric Syst* 1999;60:175–96.
 10. Boote KJ, Minguez MI, Sau F. Adapting the CROPGRO legume model to simulate the growth of faba bean. *Agron J* 2002;94:743–56.
 11. Wu L, McGechan MB. Simulation of nitrogen uptake, fixation and leaching in a grass/white clover mixture. *Grass Forage Sci* 1999;54:30–41.
 12. Thornley JHM, Bergelson J, Parsons AJ. Complex dynamics in a carbon-nitrogen model of grass-legume pastures. *Ann Bot* 1995;75:79–94.
 13. Soussana JF, Minchin FR, Macduff JH, Raistrick N, Abberton MT, Michaelson-Yeates TPT. A simple model of feedback regulation for nitrate uptake and N₂ fixation in contrasting phenotypes of white clover. *Ann Bot* 2002;90:139–47.
 14. Sinclair TR. Water and nitrogen limitations in soybean grain production I. model development. *Field Crops Res* 1986;15:125–41.
 15. Robertson MJ, Carberry PS, Huth NI, Turpin JE, Probert ME, Poulton PL, et al. Simulation of growth and development of diverse legume species in APSIM. *Aust J Agric Res* 2002;53:429–46.
 16. Evans J, O'Connor GE, Turner GL, Coventry DR, Fettel N, Mahoney J, et al. Nitrogen fixation and its value to soil N increase in lupin, field pea and other legumes in south-eastern Australia. *Aust J Agric Res* 1989;40:791–805.
 17. Schwinning S, Parsons AJ. Analysis of the coexistence mechanisms for grasses and legumes in grazing systems. *J Ecol* 1996;84:799–813.
 18. Sharpley AN, Williams JR. EPIC-erosion/productivity impact calculator model documentation. USDA Tech Bull 1990;1768:1–235.
 19. Bouniols A, Cabelguenne M, Jones CA, Chalamet A, Charpentreau JL, Marty JR. Simulation of soybean nitrogen nutrition in a silty clay soil in southern France. *Field Crops Res* 1991;26:19–34.
 20. Havelka UD, Boyle MG, Hardy RWF. Biological nitrogen fixation. In: Stevenson FJ. Ed. *Nitrogen in Agricultural Soils*, ASA, and Madison, Wisconsin, USA; 1982. p. 365–422.
 21. Vitousek PM, Cassman K, Cleveland C, Crews T, Field CB, Grimm NB, et al. Towards an ecological understanding of biological nitrogen fixation. *Biogeochemistry* 2002;57/58:1–45.
 22. Spaink HP, Wijffelman CA, Pees E, Okker RJH, Lugtenberg BJJ. *Rhizobium* nodulation gene nodD as a determinant of host specificity. *Nature* 1987;328:337–40.
 23. Roughley R, Dart P. Root temperature and root-hair infection of *Trifolium subterraneum* L. cv. Cranmore. *Plant Soil* 1970;32:518–20.
 24. Whitehead DC, Whitehead DC. Ed. *Legumes: Biological Nitrogen Fixation and interaction with grasses*, Grassland Nitrogen, CAB International, Wallingford, UK; 1995. p. 36–57.
 25. Schomberg H, Weaver RW. Nodulation, nitrogen fixation, and early growth of arrowleaf clover in response to root temperature and starter nitrogen. *Agron J* 1992;84:1046–50.
 26. Lindemann WC, Ham GE. Soybean plant growth, nodulation, and nitrogen fixation as affected by root temperature. *Soil Sci Soc Am J* 1979;43:1134–7.
 27. Richardson AC, Syers JK, Barnes RF, Ball PR, Broughman RW, Marten GC, et al. Eds. *Edaphic limitations and soil nutrient requirements of legume-based forage systems in temperate regions of New Zealand and forage legumes for energy-efficient Animal Production*, USDA, Washington; 1985. p. 89–94.
 28. Munoz N, Qi X, Li MW, Xie M, Gao Y, Cheung MY, et al. Improvement in nitrogen fixation capacity could be part of the domestication process in soybean. *Heredity* 2016;117:84–93.
 29. Saha B, Saha S, Das A, Bhattacharya PK, Basak N, Sinha AK, et al. Biological nitrogen fixation for sustainable agriculture and in agriculturally important microbes for sustainable agriculture. Springer, Singapore; 2017. p. 81–128.
 30. Sprent JI, Kozłowski TT. Water deficits and nitrogen-fixing root nodules and water deficits and plant growth. Vol. IV. *Soil water measurement, plant responses, and breeding for drought resistance*. Academic Press: New York; 1976. p. 291–315.
 31. Weisz PR, Denison RF, Sinclair TR. Response to drought stress of nitrogen fixation (acetylene reduction) rates by field-grown soybeans. *Plant Physiol* 1985;78:525–30.
 32. Weisz PR, Sinclair TR. Regulation of soybean nitrogen fixation in response to rhizosphere oxygen. II. Quantification of nodule gas permeability. *Plant Physiol* 1987;84:906–10.
 33. Sinclair TR, Muchow RC, Ludlow MM, Leach GJ, Lawn RJ, Foale MA. Field and model analysis of the effect of water deficits on carbon and nitrogen accumulation by soybean, cowpea and black gram. *Field Crops Res* 1987;17:121–40.
 34. Goh KM, Edmeades DC, Robinson BW. Field measurements of symbiotic nitrogen fixation in an established pasture using acetylene reduction and a 15N method. *Soil Biol Biochem* 1978;10:13–20.
 35. Albrecht SL, Bennett JM, Boote KJ. Relationship of nitrogenase activity to plant water stress in field-grown soybeans. *Field Crops Res* 1984;8:61–71.
 36. Engin M, Sprent JI. Effects of water stress on growth and nitrogen-fixing activity of trifolium repens. *New Phytol* 1973;72:117–26.
 37. Ledgard SF, Steele KW. Biological nitrogen fixation in mixed legume/grass pastures, *Plant Soil* 1992;141:137–53.
 38. Serraj R, Sinclair TR. Nitrogen fixation response to drought in the common bean (*Phaseolus vulgaris* L.). *Ann Bot* 1998;82:229–34.
 39. Minchin FR, Pate JS. The carbon balance of a legume and the functional economy of its root nodules. *J Exp Bot* 1973;24:259–71.
 40. Layzell DB, Rainbird RM, Atkins CA, Pate JS. Economy of photosynthate use in nitrogen-fixing legume nodules: observations on two contrasting symbioses. *Plant Physiol* 1979;64:888–91.
 41. King BJ, Layzell DB, Canvin DT. The role of dark carbon dioxide fixation in root nodules of soybean, *Plant Physiol* 1986;81:200–5.
 42. Ryle GJA, Powell CE, Gordon AJ. The respiratory costs of nitrogen fixation in soybean, cowpea, and white clover I. nitrogen fixation and the respiration of the nodulated root. *J Exp Bot* 1979;30:135–44.
 43. Twary SN, Heichel GH. Carbon costs of dinitrogen fixation associated with dry matter accumulation in alfalfa. *Crop Sci* 1991;31:985–92.

44. Warembourg FR, Roumet C. Why and how to estimate the cost of symbiotic N₂ fixation? A progressive approach based on the use of ¹⁴C and ¹⁵N isotopes. *Plant Soil* 1989;115:167-77.
45. Voisin AS, Salon C, Munier, Jolain NG, Ney B. Effect of mineral nitrogen on nitrogen nutrition and biomass partitioning between the shoot and roots of pea (*Pisum sativum* L.). *Plant Soil* 2003;242:251-62.
46. Adgo E, Schulze J. Nitrogen fixation and assimilation efficiency in Ethiopian and German pea varieties. *Plant Soil* 2002;239:291-9.
47. Lawrie AC, Wheeler CT. The supply of photosynthetic assimilates to nodules of *Pisum sativum* L. in relation to the fixation of nitrogen. *New Phytol* 1973;72:1341-8.
48. Lawn RJ, Brun WA. Symbiotic nitrogen fixation in soybeans. I. effect of photosynthetic source-sink manipulations. *Crop Sci* 1974;14:11-6.
49. Klucas RV. Studies on soybean nodule senescence. *Plant Physiol* 1974;54:612-6.
50. Nelson DR, Bellville RJ, Porter CA. Role of nitrogen assimilation in seed development of soybean. *Plant Physiol* 1984;74:128-33.
51. Jensen ES. Seasonal patterns of growth and nitrogen fixation in field-grown pea. *Plant Soil* 1987;101:29-37.
52. Khosro Mohammadi, Yousef Sohrabi, Gholamreza Heidari, Shiva Kholesro, Mohammad Majidi. Effective factors on biological nitrogen fixation. *Afr J Agric Res* 2012;7:1782-8.
53. Høgh Jensen H, Loges R, Jørgensen FV, Vinther FP, Jensen ES. An empirical model for quantification of symbiotic nitrogen fixation in grass-clover mixtures. *Agric Syst* 2004;82:181-94.
54. Watson CA, Goss MJ. Estimation of N₂-fixation by grass-white clover mixtures in cut or grazed swards. *Soil Use Manage* 1997;13:165-7.
55. Kristensen ES, Høgh Jensen H, Kristensen IS. A simple model for estimation of atmospherically derived nitrogen in grass-clover systems. *Biol Agric Hortic* 1995;12:263-76.
56. Duffy J, Chung C, Boast C, Franklin M. A simulation model of bio physiochemical transformations of nitrogen in tile-drained corn belt soil. *J Environ Qual* 1975;4:477-86.
57. Snow VO, Rotz CA, Moore AD, Martin Clouaire R, Johnson IR, Hutchings NJ, et al. The challenges-and some solutions-to process-based modelling of grazed agricultural systems. *Environ Model Softw* 2014;62:420-36.
58. Thorburn PJ, Biggs JS, Collins K, Probert ME. Using the APSIM model to estimate nitrous oxide emissions from diverse Australian sugarcane production systems. *Agric Ecosyst Environ* 2010;136:343-50.
59. Thornley JHM. Grassland dynamics: an ecosystem simulation model, CAB International, Wallingford, Oxon, UK; 1998. p. 53-7.
60. Thornley JHM, Cannell MGR. Dynamics of mineral N availability in grassland ecosystems under increased [CO₂]: hypotheses evaluated using the Hurley Pasture Model. *Plant Soil* 2000;224:153-70.
61. Thornley JHM. Simulating grass-legume dynamics: a phenomenological sub-model. *Ann Bot* 2001;88:905-13.
62. Eckersten H, Af Geijersstam L, Torssell B. Modelling nitrogen fixation of pea (*Pisum sativum* L.). *Acta Agric Scand B* 2006;56:129-37.
63. Sau F, Boote KJ, Ruiz Nogueira B. Evaluation and improvement of CROPGRO-soybean model for a cool environment in Galicia, northwest Spain. *Field Crops Res* 1999;61:273-91.
64. Hartkamp AD, Hoogenboom G, White JW. Adaptation of the CROPGRO growth model of velvet bean (*Mucuna pruriens*) Model development. *Field Crops* 2002;78:9-25.
65. Boote KJ, Minguez MI, Sau F. Adapting the CROPGRO legume model to simulate the growth of faba bean. *Agron J* 2002;94:743-56.